

PRELIMINARY AMENDMENT

Serial Number: 08/012,269

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Title: MURINE 4-1BB GENE (as amended)

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Dkt: 740.009US1

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4-1BB is a 30 kD inducible T-cell antigen, and is expressed predominantly as a 55 K dimer on both CD4⁺ and CD8⁺ T lymphocytes. The cytoplasmic tail of 4-1BB contains the sequence, Cys-Arg-Cys-Pro (SEQ ID NO:9), which is [simiJar] similar to the sequence Cys-X-Cys-Pro (SEQ ID NO:10), that mediates the binding of the CD4 and CD8 molecules to p56^{lck} a protein tyrosine kinase^{2,3}. An anti-4-1BB monoclonal antibody (53A2 mAb) was used to determine whether [4- 1BB] 4-1BB may associate with p56^{lck}. The 53A2 mAb specifically recognized 4-1BB on a CD8⁺ T-cell line, CTLL-2, and coimmunoprecipitated a 56 K protein along with 4-1BB. Peptide mapping indicated that the 56 K phosphoprotein was identical to p56^{lck}. The comimmunoprecipitation of p56^{lck} with 4-1BB also occurred in nonlymphoid cells such as insect (Sf-21) and HeLa cells when the two recombinant proteins were coexpressed. Analysis of mutant p56^{lck} recombinant proteins showed that two cysteine residues, critical for p56^{lck}-CD4 (or CD8) complex formation, are also required for the [P56^{lck}-4-1BB] p56^{lck}-4-1BB interaction. These studies establish that 4-1BB physically associates with p56^{lck}.

Please substitute page 62, second full paragraph for the paragraph in the appendix entitled "Clean Version of Page 62, Second Full Paragraph." Specific amendments to page 62, second full paragraph are detailed in the following marked-up paragraph:

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Figures 31a-c show an analysis of the association of 4-1BB and p56^{lck} in a baculoviral expression system. Figure 31a and 31b show [and] an immunoblot of 4-1BB and p56^{lck}. Sf-21 [insectcellswere] insect cells were infected with 4-1BB-, p56^{lck}-expressing recombinant baculoviruses or coinfectd with [4-1 BB] 4-1BB and p56^{lck}-expressing recombinant baculoviruses. Total lysates from Sf-21 cells infected with these recombinant baculoviruses were blotted and probed with rabbit anti-4-1BB and rabbit anti-p56^{lck} (Fig. 31a and 31b, respectively). Antigens were visualized with [alkline] alkaline phosphatase-conjugated secondary [artibodies] antibodies and chromogenic substrates, NBT and BCIP. Anti-4-1BB polyclonal rabbit serum was raised against the oligopeptide, CRPGQELTKQG (SEQ ID NO:13), which corresponds to amino acids 82 to 92 of mature 4-1BB. [figure] Figure 31c shows an immune complex kinase assay of p56^{lck}. These Sf-21 cell lysates were also incubated with isotype-matched rat IgG₁ (Fig. 31c, lane 1), 53A2 (Fig. 31c, lane 2) or anti-p56^{lck} (Fig. 31c, lane 3). The immune complexes were precipitated, subjected to the in vitro kinase reaction with [γ -³²P] and run on a 10% SDS-polyacrylamide gel as described in Fig. 30. The arrow indicates the autophosphorylated p56^{lck} proteins.

In the Claims

Please cancel claims 6-21 and 23-27 without prejudice.

Please substitute the claim set in the appendix entitled "Clean Version of Pending Claims" for the previously pending claim set. Specific amendments to individual claims are detailed in the following marked up set of claims:

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